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Navy med Res Inst
Naval Medical Research Inst., Bethesda, Md.

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QUARTERLY REPORT ON RESEARCH CONTRACT #R-38 WITH NASA,
COVERING PERIOD OCTOBER-DECEMBER 1963 [PHYSIOLOGICAL AND
PSYCHOLOGICAL MECHANISMS WHICH

DEFEND THE HUMAN BODY AGAINST HEAT AND COLD
AND DETERMINE THE EXTENT AND EFFICIENCY OF
ENERGY TRANSFORMATIONS IN THE HUMAN BODY AND IN
ISOLATED BODY CONSTITUENTS AT THE MOLECULAR LEVEL
I. HUMAN ENERGETICS -

CODE-1

(NASA CR-55562)

UNPUBLISHED PRELIMINARY DATA

Technical assistance and efforts had to be largely
diverted from human energetics to the chemosynthesis-
program, to secure important results as early as possible
in view of the still unsolved immigration status of Dr.
Kiesow. Considerable maintenance-work on the human
calorimetry-plant has become necessary and will require an
intermission in experiments on human energetics.

T.H. Benzinger [1963] 13p mfr

Lectures:

15 October 1963 THE PHYSIOLOGICAL REGULATION OF HUMAN
BODY TEMPERATURE
ONR Biological Sciences Workshop
NNMC, Bethesda, Maryland

4 November 1963 THE REGULATION OF HUMAN BODY TEMPERATURE
Naval Dental School
NNMC, Bethesda, Maryland

Publications:

- Benzinger, T.H.: "The Physiological Regulation of Human
Body Temperature. (Abstract). ONR Symposium Report
ACR-84 (1963) - page 2.
- Benzinger, T.H.: "Animal Calorimetry--Its Future" -
Transactions of ASAE, 6:116 (1963)

(NASA Order R-38)

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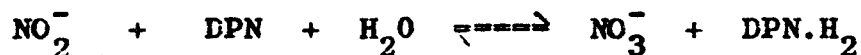
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II. MOLECULAR ENERGETICS -

(a) MECHANISM OF ELEMENTARY BIOSYNTHESIS

Report by Lutz Kiesow, M.D., D.Sc., Principal Investigator, Chemosynthesis-Project:

In the report covering period July-September 1963 the reversal of the net energy-transforming process in *Nitrobacter winogradskyi* or its particulate fractions was first described. The direction in which the net reaction



proceeded was found to be dependent on the absence or the presence and consumption of molecular oxygen. This finding was of major importance because it permitted during the first two weeks in October to clarify by means of Warburg-aufmanometry the stoichiometric relations between the oxido-reductions of the nitrate and DPN systems. It also permitted to clarify the stoichiometry of the simultaneous consumption of oxygen.

Nevertheless, the role of oxygen and the peculiar thermodynamic feasibility of the DPN.H₂-producing reaction remained obscure until another parallel, quite different series of experiments were completed: analysis with double-beam spectrophotometry revealed in nitrobacter the presence of two enzyme systems of the cytochrome-type, Cyt. I and Cyt. II, with characteristic wavelengths of maximal absorption

Cyt. I: $\lambda_1 = 588 \text{ m}\mu$, $\lambda_2 = 434 \text{ m}\mu$

Cyt. II: $\lambda_1 = 550 \text{ m}\mu$, $\lambda_2 = 413 \text{ m}\mu$

(designated Fe⁺⁺⁺ or Fe⁺⁺ in equation (2)). The reversible oxido-reductions of these two enzymes were found to be coupled with the oxido-reductions of both, the nitrate- and DPN-systems. Depending on the direction in which the transformations of the nitrate- and DPN-systems and oxygen consumption proceeded, the co-factors of the enzymes were observed to be reduced or oxidized. The combined experimental results are described and explained with the following three formulations:

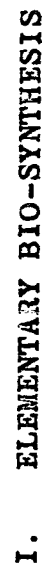
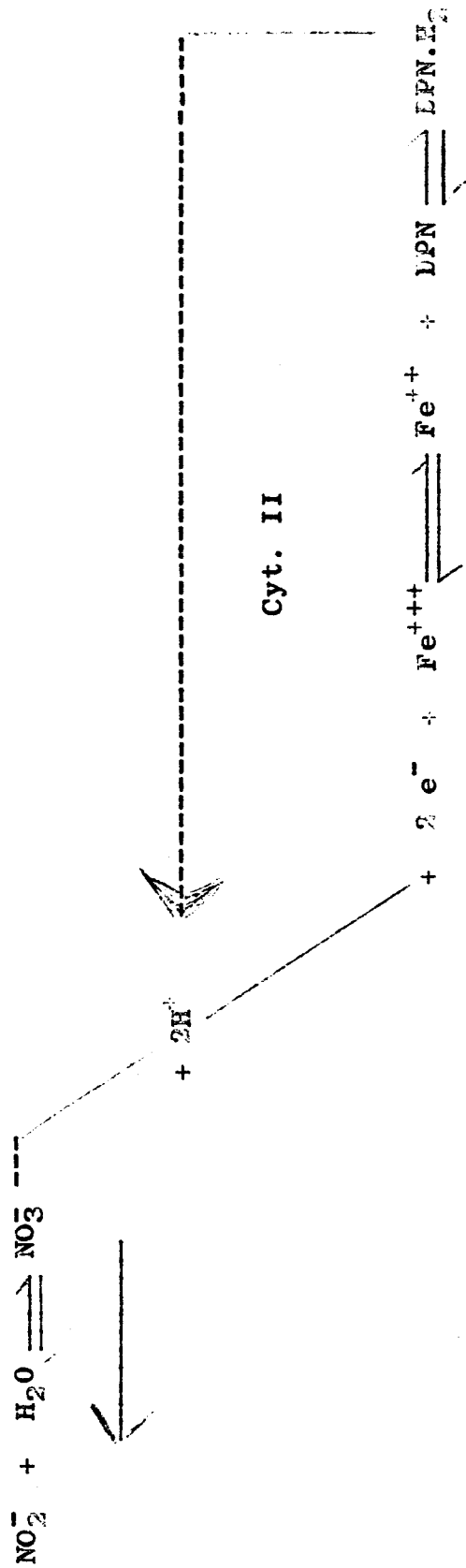


FIGURE 1. ELEMENTARY BIOSYNTHESIS -

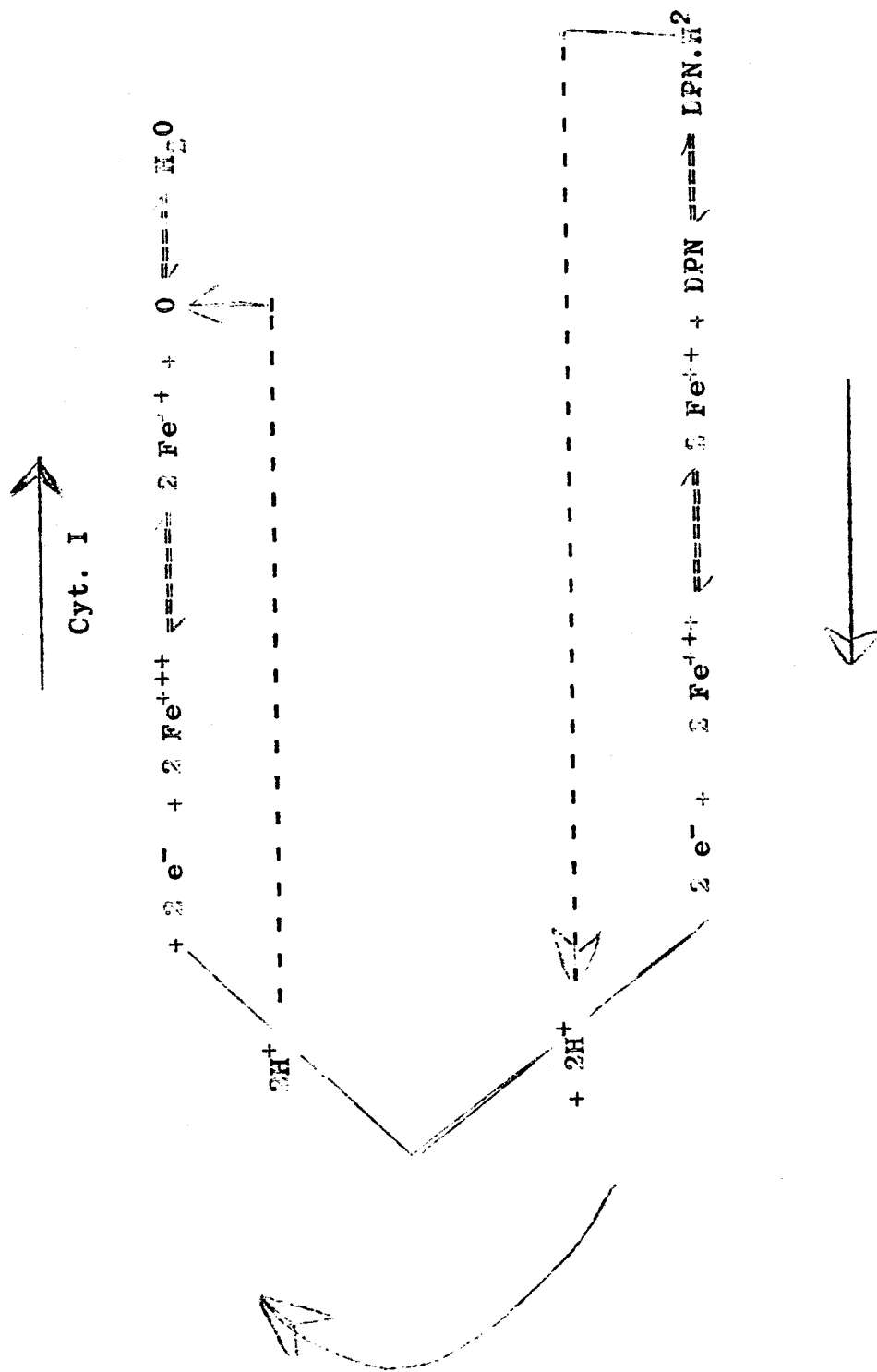
This formulation shows the mechanism of elementary biosynthesis with Nitrobacter winogradskyi in which one molecule of reduced Pyridine-nucleotide, LPN.H₂, is formed while two molecules of Nitrite, NO₂⁻, are oxidized to Nitrate, NO₃⁻, and one half molecule of gaseous oxygen, O, is consumed. An intermediary step is the temporary 'chemolysis' of water forming two protons and two electrons, which reappear as water at the end of the process. The upper right-hand equation provides the free energy or driving force. It delivers the electron required for the biosynthetic reaction proper, shown at lower right.



2. NITRATE-REDUCTION IN THE ABSENCE OF OXYGEN

FIGURE 2. BIOLOGICAL NITRATE-REDUCTION IN THE ABSENCE OF OXYGEN -

This formulation shows the process of nitrate reduction coupled with the oxidation of reduced pyridine-nucleotide. This process occurs in *Nitrobacter winogradskyi* and in particulate fractions of this microorganism, when oxygen is absent. The process is thermodynamically feasible without another, driving reaction. It is a reversal of the process of elementary biosynthesis producing, however, only one molecule of nitrate per molecule of pyridine nucleotide, $\text{LPN}\cdot\text{N}_2$, and omitting the thermodynamically unfeasible reversal of the "driving" reaction of elementary biosynthesis.



3. CELL-RESPIRATION

FIGURE 3. CELL RESPIRATION -

Cell-respiration, usually coupled with a formation of the energy donor, ATP, from ADP and inorganic phosphate in conjunction with cytochrome-oxidations, may proceed as a combination of the two reaction-branches of elementary biosynthesis. When the system that "drives" biosynthesis is run in forward gear, and the system that is driven for biosynthesis, is run in reverse in tandem, the process of cell-respiration is realized, with DPN.H_2 , reduced pyridine-nucleotide from glycolysis or Krebs-cycle-reactions, as its substrate.

It is submitted for discussion, that the operation of two basic functions of life, elementary biosynthesis and cell-respiration, by one system of enzymes would represent a mechanism of extraordinary simplicity and efficiency. It is submitted, that the mechanism described above may help in explaining certain aspects of the origin of life, and permit certain speculations concerning life on other planets.

It is submitted as a working hypothesis that any of the inorganic chemical substrates of autotrophic, chemosynthetic life, (H_2 , $S^{=}$, NH_4^+ , Fe^{++}), may be substituted for NO_2^- , to drive a similar process of elementary biosynthesis. It is submitted, also as a working hypothesis, that photosynthesis may utilize the same mechanism, with electrons mobilized by means of electro-magnetic energy on chlorophyll. Pyridine-nucleotides and cytochromes, the principal ingredients, are known to occur in photosynthetic organisms.

It remains to be shown with continuous studies on chemosynthesis under Contract #R-38, how the life-energy, harnessed in $DPN.H_2$, is utilized for the assimilation of inorganic matter: Figure 4 demonstrates, that the assimilation of radioactive carbon atoms from $H^{14}CO_3^-$ is dependent on the energy-transforming process, Figure 1.

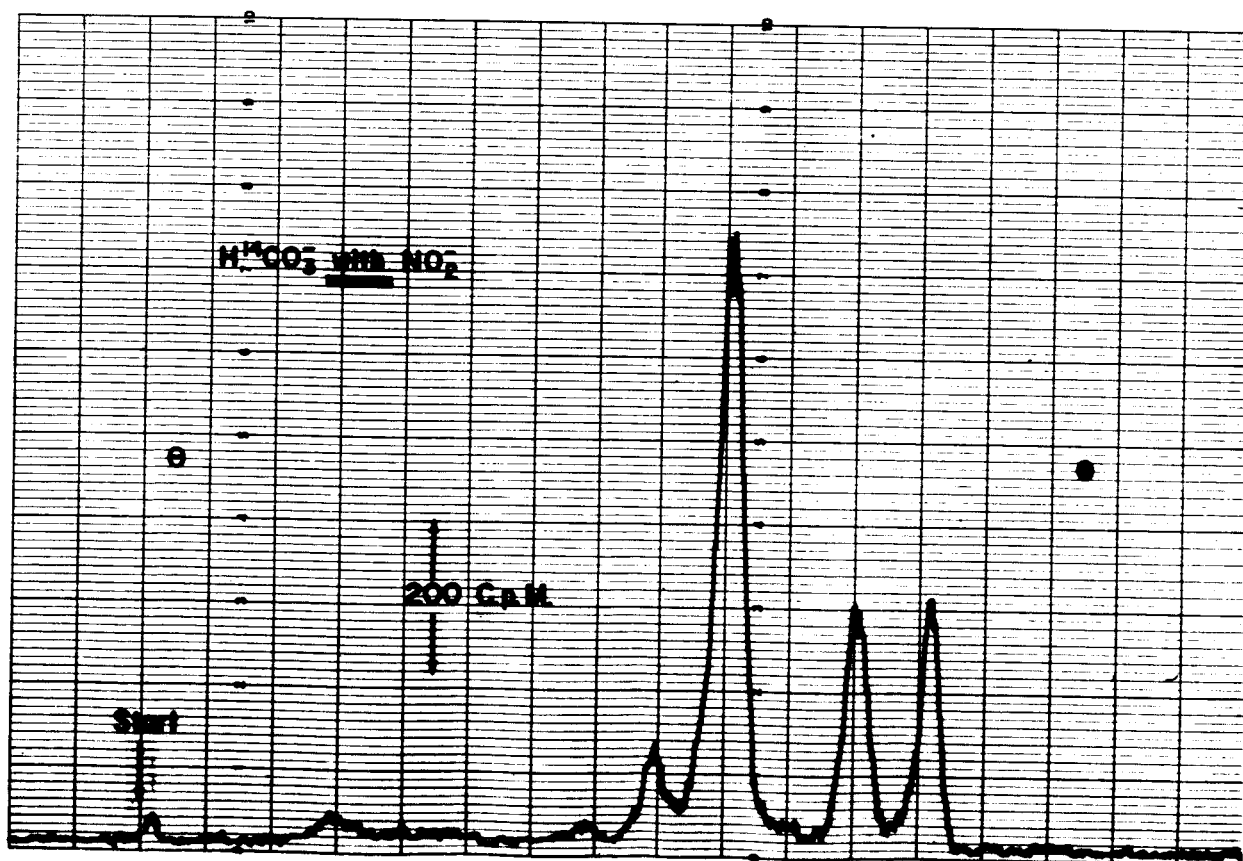
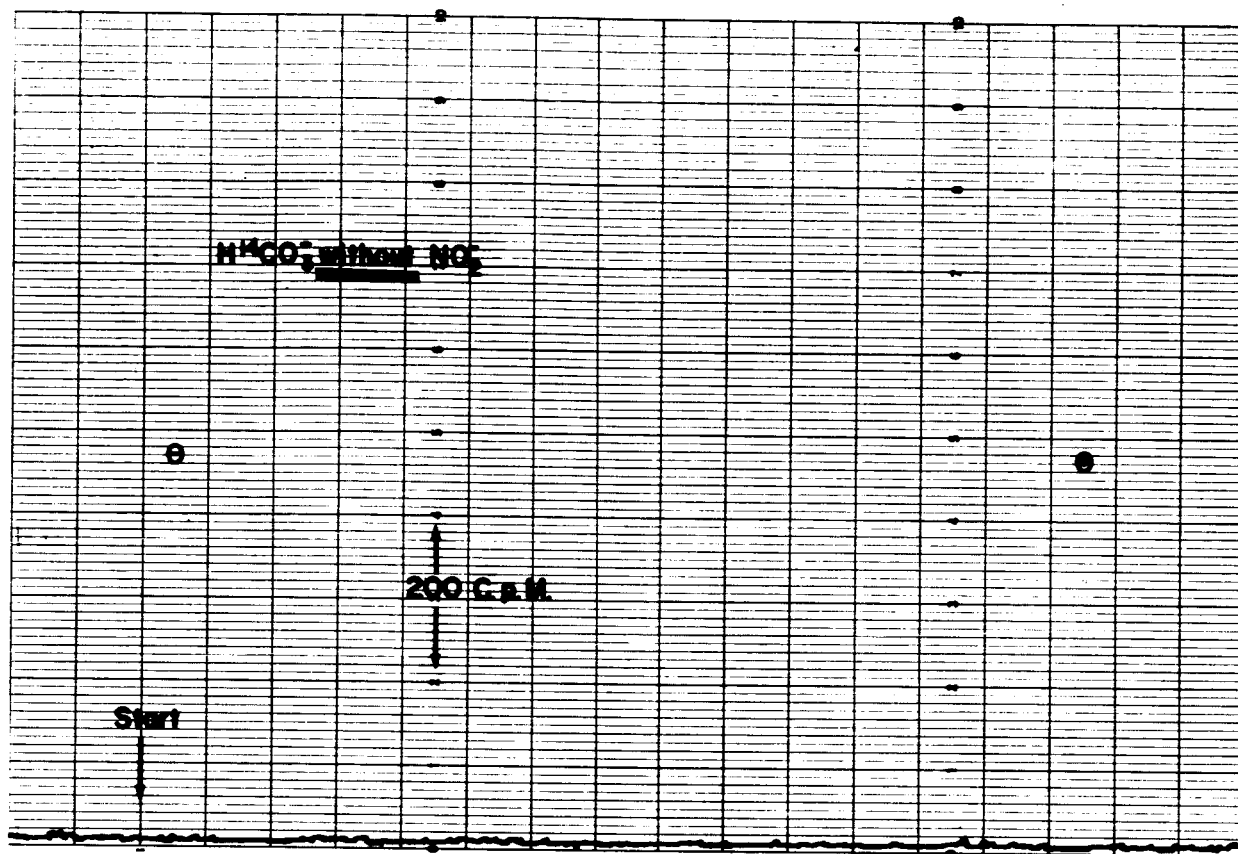


FIGURE 4. THE ASSIMILATION OF RADIOACTIVE CARBON -

Radioactive bicarbonate, $\text{NaH}^{14}\text{CO}_3$ was added to Nitrobacter in a steady state of nitrite-oxidation (below) or in the absence of nitrite (above). After 60 seconds the cells were killed and extracted with boiling methanol. Compounds were separated by ionophoresis on paper. Amount of extract corresponded to 10 microliters of packed cells. (Chemical identification of peaks will be described elsewhere).

MOLECULAR ENERGETICS (Continued)

Lectures:

- September 11, 1963 CHEMOSYNTHESIS AND ITS RELATION TO
THE SPACE SCIENCES
by Lutz Kiesow
Sixth Navy Bioastronautics Symposium
NMRI, Bethesda, Maryland
- October 15, 1963 CALORIMETRY: TOOL OF ANALYTICAL
CHEMISTRY AND CHEMICAL THERMODYNAMICS
by T.H. Benzinger
ONR Biological Sciences Workshop
NNMC, Bethesda, Maryland

Chairmanship:

Dr. Benzinger acted as Chairman of Session #6 at the
18th Calorimetry Conference in Bartlesville, Oklahoma,
October 16-18, 1963.

Publications:

- Benzinger, T.H.: "Microcalorimetry: Tool of Analytical
Chemistry and Chemical Thermodynamics" (Abstract).
ONR Symposium Report ACR-84 (1963) p. 14
- Kiesow, L. (Translation): "The Energy-Transforming Step
in Nitrobacter Chemosynthesis" - Biochem. Z. 338:400
(1963)


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Research Contract #1-38